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MULTIPLE MOLECULAR SHAPES OF LACTATE DEHYDROGENASE (LDH) IN THE LYMPHOCYTES OF FETAL AND ADULT THYMUSES AND SPLEENS

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ABSTRACT. Peripheral lymphocytes in the adult are not the only cells which have an isoenzymatic LDH profile, which has not reached complete maturation. The same is true of lymphatic cells of the thymus and the spleen.

The behavior of the isoenzymes of the lactate dehydrogenase (LDH) in the normally circulating lymphocytes of the adult human and the cells characteristic of lymphatic leukemia was described in a previous article [1]. /997*

In all the lymphatic cells of the peripheral circulatory system, both normal and pathological, five isoenzymes were isolated using electrophoretic and chromatographic methods. These isoenzymes were numbered from 1 to 5, beginning with the zone closest to the anode, and keeping the electrophoretic method as a reference.

This study was carried out on almost pure (95-100%) lymphocyte preparations, which were obtained using a method developed for these experiments [2].

In the normal lymphatic cells, the second band of activity predominates, followed by III, I, IV and V in that order.

These data show that the proteins involved in the activity of LDH in normal lymphocytes are characterized by a profile, which tends to be anodic, but which does not become completely anodized. Since one of the far bands of the LDH isoenzymes

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* Numbers in the margins indicate pagination in the foreign text.

predominates in the adult tissue cells, in contrast to the fetal ones, in which there is observed a non-differentiated profile where the third fraction predominates, the peculiar, isoenzymatic picture found in the lymphocytes might be a sign of the incomplete and indefinite maturation of some of their protein components.

The picture encountered in the isoenzymatic profile of the lymphocytic LDH might thus be a biochemical fact to support the theory, which considers the lymphocyte a totipotent cell. This element might, however, be a cell in view of its definitive, protein maturation according to the functional orientation, which it has in the organic economy.

With these assumptions, we have the opportunity to study the behavior of the isoenzymes of the LDH in the lymphocyte of fetal and adult thymuses and spleens with the aim of having the isoenzymatic picture present in the immature cells of this cell family.

As we know, the thymus is the primary organ in which cells of the lymphoid type are found, and from which they travel to the other lymphatic organs, including the spleen [3-7]

We have used electrophoretic and chromatographic methods, conducted simultaneously, whenever possible, to examine the LDH isoenzymes.

The scarcity of the cells, which are obtained in lymphocyte preparations from fetal thymuses and spleens, enabled us to make only a single electrophoretic analysis of these cells. In these cases, the percentual study of the activity, distributed among the different, isoenzymatic bands, was obtained by reading the cellogel strip on the Cromoscan densitometer. However, when it was possible to conduct the electrophoretic and chromatographic studies simultaneously, we were able to verify the exact and invariable identity of the results obtained using the two methods.

Materials and Methods

The studies were carried out on the tissue of fetal, neonatal and adult thymuses and spleens.

The fetal thymuses and spleens were obtained from fetuses aborted as a result of trauma: the age of the fetuses was 3 to 4 months. Eleven fetuses were studied.

The adult thymus, in this case, was obtained by operative biopsy, performed on subjects, whose thorax was opened for heart surgery.

The adult spleen was obtained from patients, who required sudden removal of the organ as a result of a traumatic fracture.

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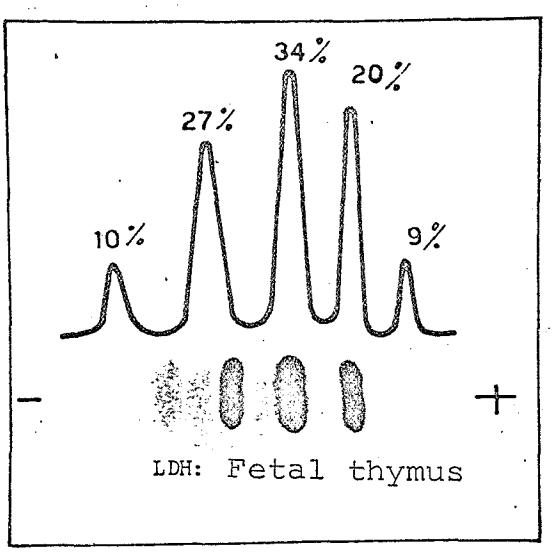

Figure 1. Electrophoretic picture of the LDH isoenzymes in the fetal thymus.

Cell Preparations

The cells of the spleen were scraped from the connective tissue with a bistoury, and were poured into a solution of 0.84% NH_4Cl so that the erythrocytes present are emolliated. After 10 minutes it is filtered through gauze and centrifuged for 10 minutes at 350 x G. The centrifuged material is then added to a 1 : 8 solution of tyrode + NH_4Cl and after five minutes is

again returned to the centrifuge at 300 x G.

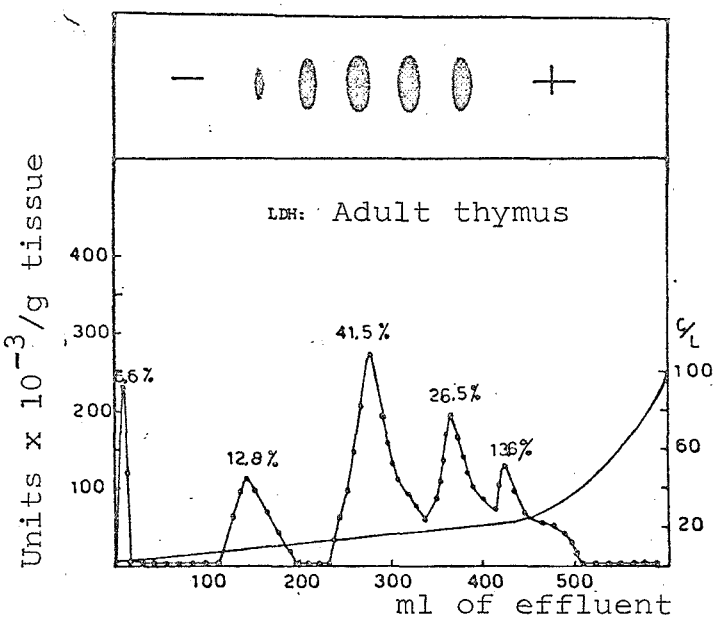

Figure 2. Electrophoretic and chromatographic pictures of the LDH isoenzymes in the adult thymus

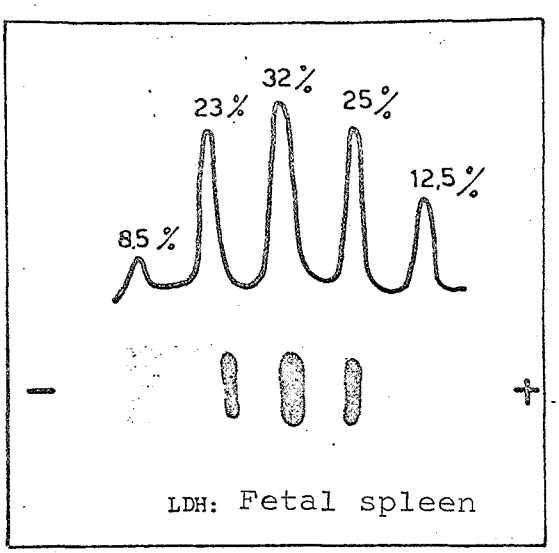

Figure 3. Electrophoretic picture of the LDH isoenzymes in the fetal spleen

The cell precipitates, which consist mainly of lymphocytes, are then successively added to liquid tyrode in a ratio of 20-30,000,000/ml for the chromatography experiments and 200,000,000/ml for the electrophoresis experiment.

The thymus was prepared by chopping it up finely and shaking it for approximately 20 minutes in a solution of 0.84% NH_4Cl in order to emolliate the few erythrocytes present.

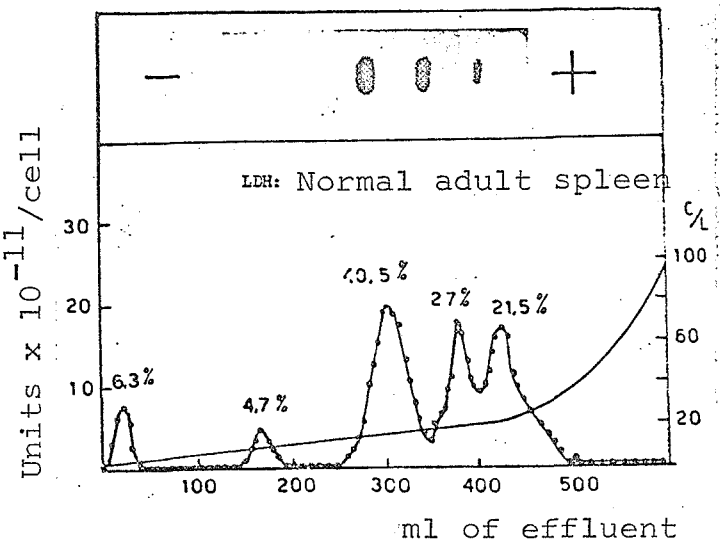

Figure 4. Electrophoretic and chromatographic picture of the LDH isoenzymes in the adult spleen

Cell Rupture

The cells of the thymus and spleen, after being homogenized in an Elvejem-Potter apparatus were quickly frozen and thawed and subjected to the effect of ultrasonic waves for twenty seconds.

The freezing, thawing and exposure to sound waves were repeated three times. Then the cells were centrifuged for 20,000 rotations in a Servall centrifuge and the residue used for the experiments after dialysis across a Tris membrane 0.005 M, pH 7.5.

Electrophoresis

The multiple molecular shapes of the LDH become apparent using the Barnett method [8] with the salts of tetrazole for cellogel (Chemitron, Milan).

The electrophoretic separation lasted for two hours using

a voltage of 10 V/cm, and a Veronal membrane 0.006 M, pH 8.6.

Chromatography /999

This test was carried out by adsorption of the sample (approximately 15 ml) on a DEAE Sephadex column, measuring 1 x 23 cm, and activated with the same solution used for the dialysis.

The experiments were carried out at 4°. The elution was done using a Tris/Hcl membrane, pH 7.5, and a concentration gradient, obtained with a nine-chamber device ("Varigrade" designed by Peterson and Sober [9]).

The final volume of the eluate is approximately 600 ml and the velocity of the flow is approximately 30 ml/hr. An automatic Radirac fraction collector with a 5 ml siphon was used.

Enzyme Dosage

The LDH was dosed using the Warburg optic test with the following final concentrations for the substrates:

- Sodium pyruvate
- NADH 1.28×10^{-4}
- Sample + Tris membrane 0.05 M, pH 7.5, at a final volume of 2.5 ml.

The data are expressed in international units per cell or per gram tissue, where unit is understood to mean the amount of enzyme which will transform 1 µM of substrate in one minute at 25°.

Results

Thymus - Figure 1 shows the distribution of the isoenzymes of LDH in the fetal thymocytes, obtained by electrophoresis. The percentual distribution of the activity in the five bands

shows a preference for the third band with practically symmetrical distribution in the other four. Therefore, this picture is typically fetal.

The amount of material obtained from the fetal thymus did not enable us to carry out a control experiment using the chromatographic method.

The adult thymus exhibits the characteristics of "free anodization", but again with preference for the third fraction. Figure 2 shows the profile and the isoenzymatic percentages obtained through chromatography, together with the electrophoretic strip.

Spleen - The fetal spleen (Figure 3) exhibits an electrophoretic profile, which is perfectly non-differentiated, and practically mirrors the data for the type [sic] of the same age.

Again for the fetal spleen cells, the scarce amount of material at our disposal made it impossible to carry out a control using the chromatographic method.

Figure 4 gives the isoenzymatic picture for LDH, obtained through electrophoretic fractionation and chromatography carried out on preparations of lymphocytes from the spleen of the normal adult. The profile tends to be anodic with preference for the third fraction.

Discussion

The LDH proteins of the thymocytes, during their lifespan, which lasts from the fetal period to adulthood, do not reach a definitive state of maturation. However, there is a tendency among these proteins toward maturation of an anodic type, with characteristics resembling the circulating lymphocytes, which exhibit an isoenzymatic profile with a tendency toward preference of the anodic bands [1].

Therefore, even the thymocytes, from the viewpoint of the proteins involved in LDH activity, never reach a definitive maturation.

The LDH isoenzymes of the spleen lymphocytes exhibit the same characteristics as those of the thymus during their entire fetal life. In the adult spleen, they assume a profile, which tends to be anodic, but not definitively mature.

It can be concluded that the peripheral lymphocytes in the adult are not the only cells, which exhibit an isoenzymatic LDH profile, which has not reached complete maturation. The same features are also exhibited by the lymphatic cells of the thymus and the spleen to an even greater degree.

These results are another fact in support of the hypothesis which attributes characteristics of pluripotentiality to the lymphatic cells, with the possibility of evolution as a function of organic necessity. In a general sense, an isoenzymatic picture with characteristics of immaturity can be interpreted as an expression of cells, which are not completely mature on a functional level.

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